Re-emergence of Sensitivity to Conventionally Used Drugs in Enteric Fever in Tertiary Care Hospital, Mymensingh

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Enteric fever is an important global public health problem in developing countries including Bangladesh. A changing antibiotic sensitivity pattern of Salmonella isolates and emergence of resistance has increased to a great concern. A total of 200 blood samples from different age and sex groups were collected for blood culture by lytic method in the Department of Microbiology, Mymensingh Medical College (MMC), Mymensingh, Bangladesh, during one year from July, 2010 to June, 2011. The present study investigates the re-emergence of sensitivity to conventionally used drugs among strains of *Salmonella typhi* and *Salmonella paratyphi A*. Among the tested antibiotics, highest number of isolates were sensitive to chloramphenicol (96.4%), followed by cotrimoxazole (92.3%), ceftriaxone (88.4%), cefotaxim (84.6%), ofloxacine (80.7%), gentamicin (76.9%), cifixime (73.1%) and azithromycin (69.2%) and least susceptible antibiotic was ciprofloxacin (38.5%) and nalidixic acid (36.4%.) Drug sensitivity in *S. typhi* and *S. paratyphi A* isolated from 26 blood culture positive cases of enteric fever was tested to determine in-vitro susceptibility pattern of prevalent strains in a tertiary care hospital. These findings suggest changing pattern of antibiotic resistance in enteric fever with reemergence of chloramphenicol and cotrimoxazole sensitivity in Mymensingh medical college and hospital, Bangladesh. MDR *S. typhi* and *S. paratyphi A* continue to be an important public health issue in Bangladesh. Hence a steady assessment of the antibiogram patterns is recommended.

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Key Words: Enteric fever, Sensitivity, Chloramphenicol, *Salmonella typhi, Salmonella paratyphi A*

Introduction

Enteric fever is a growing concern worldwide, especially in developing countries where it is endemic.[1] Antimicrobial chemotherapy against the causative agent *S. typhi* and *S. paratyphi A* emerged as an effective strategy to reduce morbidity and mortality due to the fever.[2] World Health Organization (WHO) estimates that there are about 22 million cases of illnesses and more than 216,000 deaths worldwide every year by typhoid fever during 2000.[3]

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Chloramphenicol had been the "gold standard" of therapy since its introduction in 1948. However, *S. typhi* and *S. paratyphi A* acquired resistance to chloramphenicol and other antimicrobial agents including the fluoroquinolones causing a major setback in the management of enteric fever.[4] It continues to be a major health problem despite the use of antibiotics and the development of newer antibacterial drugs.[5] If not treated properly, it carries a mortality rate of 30%, whilst appropriate antimicrobial treatment reduces the mortality rate to as low as 0.5%.[6] In cases of enteric fever, it is often necessary to commence treatment before the results of laboratory sensitivity testing.[7]

Fluoroquinolone ciprofloxacin has become the first-line drug for treatment, since the widespread emergence of *S. typhi* isolates that are multi drug resistant (MDR) to the more traditional antimicrobial agents comprising chloramphenicol, ampicillin and trimethoprim sulphamethoxazole (cotrimoxazole).[8] *S. typhi* has rapidly gained resistance to antibiotics like ampicillin, chloramphenicol and cotrimoxazole and also to previously efficacious drugs like ciprofloxacin.[9] The incidence of multidrug resistant (MDR) *S. typhi* was reported to be as high as 60 per cent.[10] A US-based study noted an increase in the number of MDR strains and nalidixic acid resistant *S. typhi* (NARST), although overall, the isolates were sensitive to ciprofloxacin and ceftriaxone.[11] Another study from Bangladesh reported a decrease in MDR isolates with no corresponding increase in sensitive strains.[12] In recent years there have been several reports indicating the re-emergence of susceptibility to drugs used in the past, such as chloramphenicol, gentamicin and cotrimoxazole.[13,14] In the present scenario of decreased clinical responsiveness of enteric fever cases to ciprofloxacin, the reintroduction of historically useful drugs in the treatment regimen of enteric fever would be of immense therapeutic importance.[14]

The aim of the present study was to analyse drug sensitivity pattern of blood culture positive cases of enteric fever. The present study was to see mainly the changing antibiogram pattern of *S. typhi* and *S. paratyphi A* with respect to re-emergence of susceptibility to conventionally used drug like chloramphenicol, gentamicin and cotrimoxazole. Reduced susceptibility of ciprofloxacin and nalidixic acid was also evaluated. Due to the variation in the susceptibility patterns of Salmonella isolates, it is important to constantly monitor the susceptibility patterns of *S. typhi* and *S. paratyphi A* to commonly prescribed antibiotics and to prevent the emergence of multi drug resistance.

Methods

This was a cross-sectional study among 200 individuals including 150 cases of clinically suspected enteric fever and 50 controls of 25 non-typhoidal illnesses and 25 healthy persons. The study was carried out in the Department of Microbiology, Mymensingh Medical College (MMC), Mymensingh, Bangladesh, during one year from July, 2010 to June, 2011. Patients of both sexes, representing all age groups were included who attended out patient departments (OPD) of Medicine and Paediatrics units of MMC Hospital, irrespective of their prior antibiotic treatment.

The aseptically collected 200 blood samples were collected in heparinized tube containing lytic solution which was made in our laboratory for initial processing. Blood sample were mixed with lytic solution and mixture were shacked, vortexed and centrifuged at 3000 rpm for 30 minutes. After centrifugation, the supernatants were discarded and 1ml of the deposit containing

the pathogen is vigorously vortexed and entire sediment were directly inoculated onto blood agar and Mac Conkey agar media. The inoculated culture plates were immediately placed in an incubator at 37^0C for 24 - 48 hours. If there was growth by lytic centrifugation method, colonies of *S. typhi* and *S. paratyphi A* were further identified by standard laboratory techniques.[15,16]

Antibiotic sensitivity testing: Antibiotic susceptibility patterns of the 26 blood culture positive cases of enteric fever were determined by the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar according to Clinical and Laboratory Standards Institute (CLSI) guidelines and interpretative criteria.[17] All media, antibiotics and biochemicals were obtained from Hi Media Lab. Pvt. Ltd., Mumbai, India. Antibiotics discs used were chloramphenicol 30 mcg/disc, ampicillin 10 mcg/disc, ciprofloxacin 5 mcg/disc, ceftriaxone 30 mcg/disc, cotrimoxazole 25 mcg/disc, cefotaxim 30mcg/disc, cifixime 5 mcg/disc, nalidixic acid 30 mcg/disc, gentamycin 10 mcg/disc, and ofloxacin 5 mcg/disc, azithromycin 15 mcg/disc. Commercially available, six mm discs (Hi Media) were used. *Salmonella typhi* collected from department of microbiology, BSMMU was used as control. The inocula were prepared by picking three to five well-isolated colonies of the same morphological type into broth and adjusting to obtain turbidity optically comparable to that of the 0.5 McFarland standards. Minimum inhibitory concentrations (MICs) were not measured.

Results

The present study was conducted on a total 200 individuals of different age and sex, including 150 cases of clinically suspected enteric fever and 50 controls of 25 non-typhoidal illnesses and 25 healthy persons. Among the 150 suspected cases of enteric fever, almost all of the cases complained of fever (148, 98.7%) followed by headache (52, 34.7%), anorexia, nausea and vomiting (39, 25.8 %), abdominal discomfort with diarrhoea (34, 22.6 %) and constipation (29, 19.3%) for 3-10 days (Figure 1)

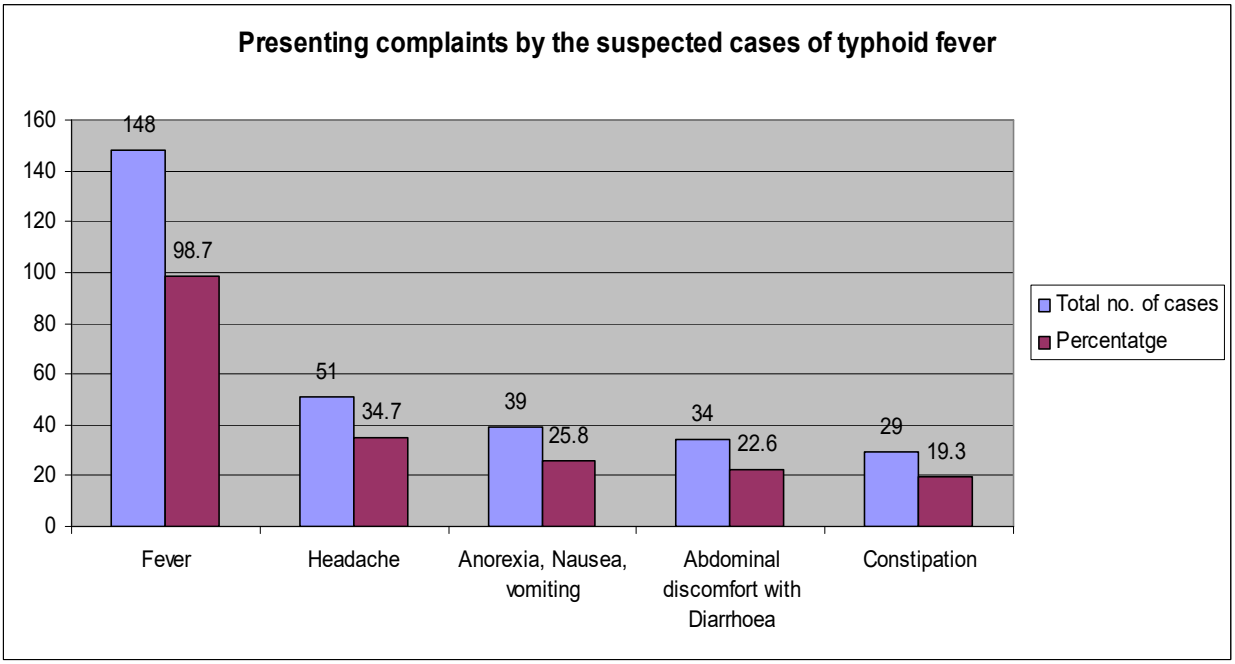


Fig 1. Presenting complaints by the suspected cases of enteric fever

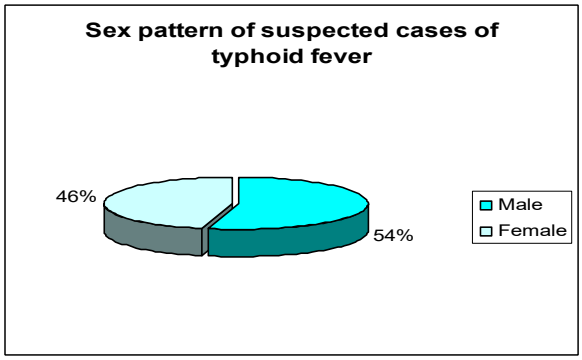
Age range of the study population was from 2 years to 60 years, mean age was 18.6 years. Age distribution of 150 suspected cases and 50 controls showed that the majority of the respondents of the cases (43, 28.6%) belonged to the age group of 1 to 5 years followed by (34, 22.7%) to the age group of 16 -20 years and (32, 21.3%) to the age group of >30 years (Table-I).

Table I: Age group distribution of suspected cases of enteric fever

Age in years	No of cases (%)
1 - 5	43 (28.6%)
6 - 10	14 (9%)
11 - 15	8 (5.3%)
16 - 20	34 (22.7%)
21 - 25	12 (7.7%)
26 - 30	7 (4.7%)
>30	33 (21.3%)

Male female ratio 1:1.2

Distributing the sex of study population showed that total males were 81(54%) and female 69(46%) in the cases group and corresponding values for controls were 26 (52%) and 24(48%) respectively. Male to female ratio of cases were 1.2:1(Figure-2).



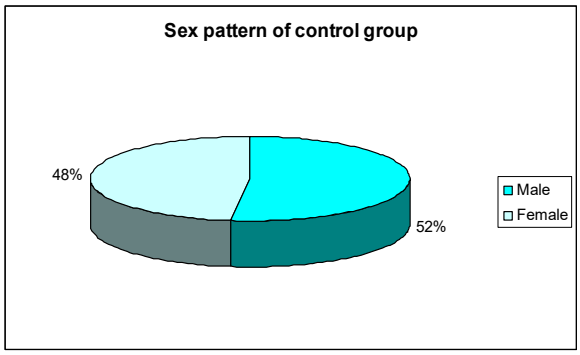


Fig 2. Sex pattern of study population

Among 150 blood samples from the suspected cases, 23 were positive for *S. typhi* and 3 were positive for *S. paratyphi A* by lytic blood culture method. None of the healthy controls was positive by lytic blood culture method(Table-II).

Table II: Isolation and identification by lytic blood culture method

Subject	Organism isolated	
	S. typhi	*S. paratyphi A*
Suspected Cases (n=150)	23	03
Controls (n=50)	0	0

Antimicrobial susceptibility pattern of Salmonella isolates (n=26) showed that the highest number (96.1%) of isolates were sensitive to chloramphenicol, followed by cotrimoxazole (92.3%), ceftriaxone (88.4%), cefotaxim (84.6), ofloxacine (80.7), gentamicin (76.9%), cifixime (73.1%)and azithromycin (69.2%) (Table III).

Table III: Antibiotics sensitivity patterns of total Salmonella isolates (n=26)

Antibiotics	Resistance No (%)	Intermediate No (%)	Sensitive No (%)
Ampicillin	10(38.5)	5(19.2)	11(42.3)
Cotrimoxazole	2(7.6)	0(00)	24(92.3)
Chloramphenicol	0(00)	1(3.8)	25 (96.1)
Azithromycin	7(26.9)	1(3.8)	18(69.2)
Ofloxacin	5(19.2)	0(00)	21(80.7)
Ciprofloxacin	12(46.1)	4(15.3)	10 (38.5)
Nalidixic acid	19(73.1)	0(00)	9(34.6)
Gentamicin	0(00)	6(23.1)	20(76.9)
Cifixime	7(26.9)	0(00)	19(73.1)
Cefriaxone	1(3.8)	2(7.6)	23(88.4)
Cefotaxime	2(7.6)	2(7.6)	22(84.6)

Highest numbers of isolates (73.1%) were resistance to nalidixic acid, followed by Ciprofloxacin (46.1%) resistance was also high. In five patients of *S. typhi,* though the in-vitro studies showed sensitivity to ciprofloxacin, there was no clinical response for up to a week. The isolates from these patients were also sensitive to chloramphenicol and the patients improved with this drug.

Discussion

Enteric fever continues to be a major public health problem in our country. It rapidly develops resistance to various drugs makes the situation more alarming. Wide variations in different geographical regions in Bangladesh make it necessary to ensure the sensitivity of the enteric fever pathogen to antibiotics before instituting therapy.

Chloramphenicol was the first antibiotic which was used against typhoid and resistance to this drug developed in the seventies and eighties and thereafter, to the other first line drugs like trimethoprim, sulfamethaxazole and ampicillin. In the nineties, increasing resistance to ciprofloxacin which was a good alternative for the multidrug resistant enteric fever pathogen emerged in both *S. typhi* and *S. paratyphi A.*[18] Resistance to ceftriaxone has also been reported.[19]

In the present study, 200 purposefully selected individuals were investigated including 150 clinically suspected cases of enteric fever and 50 controls (25 non-typhoid febrile illnesses and 25 healthy individuals). The disease affected all ages; however most of the cases (28%) of the study were in the age group of 1–5 years. These findings correlate with the observation made by Saha and associates who found that children between 2-3 years of age are most susceptible age group (35.6%).[20] Almost similar study done by Brooks and others showed that the prevalence of typhoid fever in children of under 5 years were much higher than other age group.[21] The child aged group < 5 years were more prone to infection, which may be due to a lack of immunity transferred by mothers' milk or the non consumption of potable drinking water as is the common practice in rural areas.[20]

In present study, among 150 clinically suspected enteric cases 54% were male and 46% were female. Another study done by Butler and others also showed similar result that infection rate was slightly higher in male.[22] Butler expressed his opinion that greater exposure of male to contaminated food and water out side the home might be

region of higher rate of infection among this population.

In this study, the majority (96.1%) of isolates were sensitive to chloramphenicol, followed by cotrimoxazole (92.3%). There have been some reports of the reemergence of the sensitivity of *S. typhi* to chloramphenicol and other first line drugs. The National *Salmonella* and *Escherichia* Centre (NSEC), Central Research Institute, Kasauli, India, has provided a service to the nation and showed that the susceptibility of salmonella isolates to chloramphenicol, ampicillin, and trimethoprim was 95.3%, 94.5%, and 94.5%, respectively (14). Bhatia and others reported that the highest (96%) sensitivity of *S. typhi* to chloramphenicol in India.[23] Another study by Yashavanth and Vidyalakshmi found that the re-emergence of chloramphenicol (97.4%) sensitivity among the strain of *S. typhi* pathogens in Mangalore in 2007.[24]

In present study, some isolates of *Salmonella* were found resistant to ciprofloxacin (46.1%) and nalidixic acid (73.1%). Nalidixic acid-resistant (NAR) *S. typhi* with decreased susceptibility to ciprofloxacin is now endemic in India and neighbouring countries, constituting a threat to global health.[25,26,27] In a study by Ali et al. showed that least susceptible antibiotic for enteric fever was ampicillin 62.5% and nalidixic acid 95%.[13] Another study from Bangladesh showed that the decreased susceptibility to ciprofloxacin was detected in 24 (18·2%) out of 132 randomly selected strains during 1990–2002.[28] Fluoroquinolones, especially ciprofloxacin, have been in use for more than 18 years and have remained an important weapon against *S. typhi*. In spite of this, in recent years, several reports have appeared worldwide concerning reduced activity of ciprofloxacin against *S. typhi.*[25,26,27,29] In perspective of Bangladesh, ciprofloxacin is commonly used in case of enteric fever may be due to ciprofloxacin continues to be the mainstay in the treatment of enteric fever as it is orally effective and economical.

In five patients of *S. typhi,* though the in-vitro studies showed sensitivity to ciprofloxacin, there was no clinical response up to a week. This observation is in accordance with the findings of Chandel et al., who reported that treatment failures did not always correlate with higher MICs to nalidixic acid and ciprofloxacin alone.[25] Their findings suggest that, despite prolonged doses of ciprofloxacin, treatment failures are common with isolates sensitive to ciprofloxacin and nalidixic acid.

In this study, 3.8% of isolates of *S. typhi* were found resistant to ceftriaxone. Third generation cephalosporins have been recommended as an alternative to quinolone treatment in enteric fever and several physicians have claimed good results with them, particularly with ceftriaxone.[30] As consequences of extensive use of ceftriaxone and other third generation cephalosporin, resistance is being reported with increasing frequency all over the world. Another study by Saha and others reported about the highly ceftriaxone resistant strain of *S. typhi* in Bangladesh.[31]

The findings of the present study indicated that the first line antibiotics like chloramphenicol, cotrimoxazole may still have a role in the treatment of enteric fever. Constant surveillance and antibiotic sensitivity testing is required from different geographical areas in the country, to keep abreast with emerging patterns of drug sensitivity in enteric fever. So this study indicates that chloramphenicol, cotrimoxazole can be used as a first line therapy and ciprofloxacin, nalidixic acid should be avoided for treatment.

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